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ORIGINAL ARTICLE

Challenge of Frequency of *blaZ* gene among *Staphylococcus epidermidis* Harboring *mecA* gene Isolated from Clinical Samples in Iraq's Al-Basrah Governorate

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ABSTRACT

Background: *Rapidly identifying Staphylococcus epidermidis isolates from various types of coagulase-negative staphylococci (CoNS) is essential, but identifying resistant agents can also significantly enhance existing diagnostic and treatment approaches.* ***Objectives:*** *To identify the patterns of β-lactam antibiotic sensitivity and methicillin resistance, and identify mecA and blaZ in S. epidermidis isolates from clinical specimen collections in the Iraqi province of Al-Basrah.* ***Methodology:*** *The current study detected one hundred isolates of coagulase-negative staphylococci (CoNS). A variety of clinical samples, such as blood, urine, skin, surgical wounds, and tracheal and ocular swabs, were used to collect these isolates. The isolates were identified as coagulase-negative staphylococci (CoNS). Identification was confirmed using the Vitek®2 technology and standard biochemical assays. Using PCR and certain primers, the β-lactamase gene blaZ and the Methicillin resistance gene (mecA) were discovered.* ***Results:*** *The isolates of S. epidermidis showed the highest sensitivity to Cephalexin (64.1%) and the highest resistance to Penicillin (87.2%). According to agar screening, 51.3% of S. epidermidis isolates gave positive results for Methicillin-resistant. Of the 39 S. epidermidis isolates that were subjected to PCR analysis, 82.1% of respondents obtained positive results for the blaZ gene and 59% for the mecA gene.* ***Conclusions****: Prevalence of mecA gene and blaZ gene between the S. epidermidis isolates gave the alert to increase the virulence of S. epidermidis, also PCR showed more accurate results to detection of mecA gene and blaZ gene in clinical isolates.*

INTRODUCTION

For a long time, it was believed that coagulase-negative staphylococci (CoNS), namely *Staphylococcus epidermidis*, were an essential part of the normal flora present in all human body parts, such as the nares, head, and axilla. Their presence was thought to be necessary for the maintenance of healthy skin. *S. epidermidis* is now recognized among the most frequent reasons for nosocomial and implant-associated infections[1,2]. Blood infections and nosocomial infections are frequently caused by *S. epidermidis*. Numerous illnesses, including bacteremia, pneumonia, wounds and skin infections, endocarditis, urinary tract infections, and soft tissue infections, have been linked to it[3] .

Penicillin was once the most common therapy for treating Staphylococcus infections; however, since 1968, Penicillin resistance has increased significantly in CoNS[2,4]. Penicillin resistance in staphylococci is conferred by two processes. The primary and most important mechanism is the formation of β-lactamase, which hydrolyzes Penicillin's β-lactam ring, rendering it useless[5,6]. The latter is primarily linked to human isolates and exhibits resistance because *mecA* codes for the penicillin-binding protein PBP2a[7].

It has also been demonstrated that *blaZ* causes Coagulase-negative Staphylococci (CoNS) to be resistant to Penicillin, proving that blaZ is one of the primary pathways allowing staphylococci to become resistant to penicillin[7]. Staphylococcus PBP2a expression, which is encoded by the *mecA* gene, results in methicillin resistance[8]. PBP2a, a protein that binds to penicillin, has a poor affinity for methicillin and other β-lactam antibiotics, which is why sensitive staphylococci do not have this protein[9]. These genes may be transmitted through the central nervous system (CNS), as evidenced by studies of resistance gene transfer between CoNS and *S. aureus*. This means that, given a chance bacterial condition of use, staphylococci of various species may exchange *mecA* and *blaZ* in the same habitat[6,10].

CoNS are important reservoir for mobile genetic elements that give resistance to Tetracyclines, Aminoglycosides, Quinolones, β-lactams, and macrolides[10]. To become resistant to a wide range of drugs, these microbes employ a number of antibiotic resistance techniques. These tactics consist of changing the targets of antibiotics, producing enzymes that deactivate medications, and lowering the concentration of antibiotics inside cells[11,12].

Therefore, the aim of current study is to identify the Methicillin resistance and β-lactam antibiotic susceptibility pattern, and identify *mecA* and *blaZ* in *S. epidermidis* isolates from clinical samples in the Iraqi province of Al-Basrah.

METHODOLOGY

Bacterial Isolates

From October 2023 to January 2024, a total of 100 of (CoNS) isolates have been collected from a variety of clinical specimens (including skin infections, eye, surgical wounds, tracheal, blood and urine), in the Al-Basrah province Iraq. According to Sharma et al.[13] urease, catalase, tube coagulase, and mannitol salt agar growth were among the common bacteriological methods used to identify the *S. epidermidis* isolates. The validated identification was the second step completed by the Vitek®2 system (Vitek®2 GP ID-P Reference number 21342, bioMérieux, USA). The isolates were kept in brain heart infusion (BHI) medium with 15% glycerol added at -20°C.

Antibiotic susceptibility pattern of β-lactam antibiotics

The Kirby-Bauer disc diffusion method was used to assess the pattern of antibiotic susceptibility to β-lactam antibiotics. The antibiotics that were assessed were Penicillin (10 µg), Ceftriaxon (30 µg), Amoxicillin (10 µg), Cefoxitin (30 µg), Cefotaxime (30 µg), Cephalexin (30 µg), and Cephazolin (30 µg)[3].

Detection of Methicillin-resistant

Cefoxitin diffusion disc method

Mueller-Hinton agar plates (LAB-media, England) and antibiotic disks Cefoxitin (30 µg) were used to test for *S. epidermidis* isolates in compliance with CLSI standards in order to determine the sensitivity pattern[14,15].

Oxacillin agar screen method

According to NCCLS guidelines[16], an oxacillin agar screen plate (Mueller-Hinton agar supplemented with 4% NaCl and 6 µg of oxacillin per ml) and micro-broth dilution were used to look for *S. epidermidis* isolates.

DNA extraction

The isolates' genomic DNA was separated using the Wizard® Genomic DNA Purification Kit (Promega, USA).

mecA and *blaZ* genes Detection

According to *Lina etal*, *and Shamansouri etal*, methods[17,18] respectively, the polymerase chain reaction has been utilized to discover the β-lactamase resistant *blaZ* gene and the methicillin-resistant *mecA* gene in *S. epidermidis* isolates. The primers that were used to amplify the *blaZ* and *mecA* genes were listed in (Table1).

Table 1: Specific primers of the *mecA and blaZ* genes used in PCR

Primers	Sequence	Length	Size (bp)	Optimizing Ta*
mecA-F	5'-AAAATCGATGGTAAAGGTTGGC-3'	22	533	53°C
mecA-R	5'-AGTTCTGGAGTACCGGATTTGC-3'	22		53°C
lukS-PV	5'-ATCATTAGGTAAAATGTCTGGACATGATCC A-3'	27	433	50ºC
lukF-PV	5'-GCATCAACTGTATTGGAGCAAAAGC-3'	21		50ºC

* Ta: Annealing temperature.

RESULTS

The 86 isolates of coagulase-negative staphylococci (CoNS) isolates were collected between October 2023 and January 2024. The CoNS isolates isolated from 32 (37.2%) urine, 20 (23.3%) blood, 14 (16.3%) skin infections, 12 (14%) surgical wounds, 5 (5.8%) tracheal and 3 (3.4%) eyes. Vitek®2 and biochemical tests have been used to identify bacterial growth, and the results were as follows: *Staphylococcus epidermidis*, 39 (45.3%), *Staphylococcus haemolyticus*, 28 (32.5%), and *Staphylococcus saprophyticus*, 11 (12.8%), were the most prevalent types of bacteria, followed by *Staphylococcus warneri*, 4 (4.7%), *Staphylococcus schleifericus*, 3 (3.5%), and *Staphylococcus capitis*, 1 (1.2%) (Figure1).

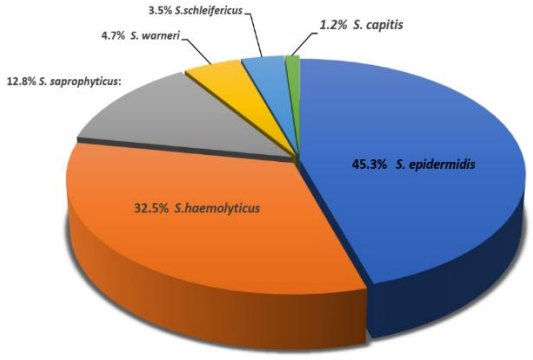

Fig. 1: The frequency of coagulase-negative staphylococci (CoNS) isolates

Distribution of 39 *S. epidermidis* isolates in different clinical specimens, in the current study, were as follows: the majority of isolates have been identified in urine 15 (38.5%) and blood 9 (23.1%), followed by skin infections 7 (17.9%) and surgical wounds 5 (12.8%), while the lowest percentage of isolates were found in tracheal 2 (5.1%) and eye samples 1 (2.6%) (Figure2).

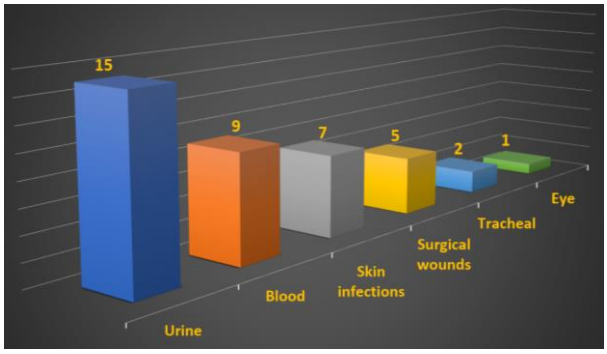

Fig.2: *Staphylococcus epidermidis* distribution in clinical samples.

Additionally, the Kirby-Bauer disc diffusion technique was used to investigate the antibiotic susceptibility pattern of β-lactam drugs. The *S. epidermidis* isolates showed greater sensitivity to Cephalexin 25 (64.1%) compared to Cephazolin 22 (56.4%) and greater resistance to Penicillin 34 (87.2%) compared to Ceftriaxone 31 (79.5%) (Table2).

Furthermore, out of n=39 *S. epidermidis* isolates tested for detection of Methicillin-resistant by using Cefoxitin diffusion disc method, the 20 (51.3%) *S. epidermidis* isolates showed positive results for Methicillin-resistant, while 19 (48.7%) *S. epidermidis* isolates gave negative results. Whereas, using oxacillin agar screen method gave 17 (43.6%) *S. epidermidis* isolates as positive for Methicillin-resistant, and 22 (56.4%) *S. epidermidis* isolates gave negative results (Table3).

Table 2: β-lactam antibiotics sensitivity pattern *Staphylococcus epidermidis* isolates

No.	Antibiotic	*Staphylococcus epidermidis n=39*		
		Resistant	**Intermediate**	**Sensitive**
1	Penicillin	34(87.2%)	0	5(12.8%)
2	Ceftriaxone	31(79.5%)	2(5.1%)	6(15.4%)
3	Amoxicillin	27(69%)	0	12(31%)
4	Cefoxitin	24(61.5)	4(10.3%)	11(28.2%)
5	Cefotaxime	21(53.8%)	1(2.6%)	17(43.6%)
6	Cephalexin	11(28.2%)	3(7.7%)	25(64.1%)
7	Cephazolin	16(41%)	1(2.6%)	22(56.4%)

Table 3: Methicillin sensitivity pattern for *Staphylococcus epidermidis* isolates using conventional methods and PCR Technique

Parameter	*Methods used for detection of MRSA*		
	Cefoxitin diffusion disc method	**Oxacillin agar screen method**	**PCR Technique**
Methicillin resistance *S. epidermidis*	20 (51.3%)	17 (43.6%)	23 (59%)
Methicillin sensitive *S. epidermidis*	19 (48.7%)	22 (56.4%)	16(41%)

PCR results of amplified *blaZ* gene showed 32(82.1%) *S. epidermidis* isolates had gave positive results for *blaZ* gene, while 7(17.9%) *S. epidermidis* isolates, had gave negative results for *blaZ* gene. Furthermore, the amplified *mecA* gene results revealed that 16 (41%) of the *S. epidermidis* isolates had negative *mecA* gene results, whereas 23 (59%) of the isolates had positive *mecA* gene results.

DISCUSSION

Coagulase-negative staphylococci, or CoNS, are an opportunist microbial flora that has become increasingly important in nosocomial infections in recent years. Due to nosocomial infections are largely caused by coagulase-negative staphylococci (CoNS), accurate detection of bacterial strains in laboratories is essential, due to antibiotic resistance is increasing and infections

are becoming more resistant to medication, that needs more improved diagnosis and more efficient antimicrobial therapy, particularly in hospital settings[6]. The CONS strains are often reported as the cause of nosocomial infections. They are common as mucous and skin membrane pathogens. Methicillin resistance in particular poses a difficulty in the therapeutic management of these infections[5].

The results in our current study showed that among 39 *S. epidermidis* isolates, the highest percentage were from urine (38.5%) and blood samples (23.1%). Other studies[3,19,2] also reported the highest isolation were also from urine and blood samples. The combined findings of these three investigations support the findings of the present investigation. It is necessary to look at the increasing frequency of β-lactam resistance in *S. epidermidis* isolates. Based on phenotypic testing, the current study's findings indicated that 87.2% and 79.5% of the *S. epidermidis* isolates were resistant to Ceftriaxone and Penicillin. On other hand, our results revealed that among the 39 *S. epidermidis* isolates in the current study, (82.1%) contained *blaZ*, which is in agreement with other investigators[3,21,22]. In addition, Du et al.[20] found that resistance to Cephalexin and Ciprofloxacin was the lowest, whereas resistance to Penicillin and Ceftriaxone was the highest. Also, the resistance to Cephalexin and Cephazolin was the lowest, while the resistance to Penicillin, Methicillin, Ceftriaxone, and Ceftizoxime was the highest[22].

MRSA is currently one of the most prevalent organism isolated from nosocomial infections. The implementation of an antibiotic treatment regimen and early MRSA detection are crucial[23,24]. *S. epidermidis* resistance to Methicillin has increased globally[25], possibly due to the transfer of a *mecA* gene from the species to *S. aureus* through horizontal gene transmission[26]. About 70% of the *S. epidermidis* isolates in our investigation had the *mecA* gene. Other studies[27,28,29]**.** reported the harbored of *mecA* gene among the *S. epidermidis* in different percentage (64.0%,75.43% and 70.7%), respectively. Several investigations revealed that *S. epidermidis* had the *mecA* gene and had significant rates of 95.8% and 93.75%. According to studies by Najar *et al.*[32] and Dos Santos *et al.*[33], the *mecA* gene was present in 85% and 92.2% of isolates, respectively. Other studies by Wang *et al.*[34] and Behshood, *et al.*[2] reported a low of *mecA* gene existence, the (34.4% and 10%) of isolates harbored *mecA* gene respectively.

The results of PCR analysis in the current study confirmed the presence of *blaZ* gene in 32 (82.1%) *S. epidermidis* isolates, while the 7(17.9%) *S. epidermidis* isolates gave negative results for *blaZ* gene, and these results were similar with the study of Fowoyo and Ogunbanwo[3], study of Al-Amara[11] discovered that the *blaZ* gene was present in 149 of the 198 specimens (75.25%). Resistance among isolates to different β-lactam antibiotics is increasing. As a result, estimation is necessary for the treatment of illnesses linked to *S. epidermidis* infections[3,7,20]. Additionally, our results of the amplified *mecA* gene indicated that 23 (59%) of the isolates of *S. epidermidis* had positive *mecA* gene results, whereas the *mecA* gene test results for 16 (41%) *S. epidermidis* isolates were negative. Freney *et al.*[12] study was published showed that Methicillin-resistant coagulase-negative staphylococci (MRCoNS) accounted for 15 (53.57%) of the 28 CoNS isolates, while Methicillin-sensitive coagulase-negative staphylococci (MSCoNS) accounted for 13 (46.43%). According to the Pillar *et al.*[35] study, MRSA was detected in 55.7% of inpatients and 48.7% of outpatients.

CONCLUSION

Prevalence of *mecA* gene and *blaZ* gene between the *S. epidermidis* isolates gave the alert to increase the virulence of *S. epidermidis*. Also, the use PCR technique gave the more accurate results for detection of *mecA* gene and *blaZ* gene in clinical isolates and prevents the therapeutic failure in hospitals.

Ethical approval

The Ethical Committee of the College of Science, Department of Pathological Analysis, University of Basra approved this work under No. 37/24/2577 dated September 21, 2023.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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